Faculty of Resource Science and Technology

DETECTION OF HAZARDOUS HETEROCYCLIC HYDROCARBON FROM WATER SAMPLES

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Detection of Hazardous Heterocyclic Hydrocarbon from Water Samples

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DECLARATION

I declared that this project entitled "Detection of Hazardous Heterocyclic Hydrocarbon from Water Samples" is the result of my own research except as cited in the references. This project has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

Signature: ....

Name : YEAP KIM HENG

Date : 2 JULY 2013

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LIST OF ABBREVIATION

HOADA	-	2-Hydroxy-6-oxo-(2'-aminophenyl)-hexa-2E,4Z-dienoate
HOPDA	-	2-Hydroxy-6-oxo-6-phenylhexa-2,4-dienoate
PAH	-	Polycyclic Aromatic Hydrocarbon
TSA/V	-	Total Surface Area / Volume
nm	-	nanometer
HPLC	-	High Performance Liquid Chromatography
GC	-	Gas Chromatography
FID	-	Flame Ionization Detector
HCl	-	Hydrochloric acid
NaOH	-	Sodium Hydroxide
g	-	gram
ml	-	millilitre
DMSO	-	Dimethyl Sulphoxide
ONR7a	-	artificial sea water
r.p.m.	-	rotation per minute
μL	-	microliter

$MgCl_2$	-	Magnesium Chloride
K	-	Potassium
Mg	-	Magnesium
°C	-	Degree Celcius

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Detection of Hazardous Heterocyclic Hydrocarbon from Water Samples

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ABSTRACT

Environmental pollution is getting more severe with the contaminations caused by chemicals such as heterocyclic hydrocarbons from oil spills, industrial activities and the use of chemicals which reside in the environment for extensive period of time. In this study, the feasibility of detection method for hazardous heterocyclic hydrocarbon using degrading bacteria in water sample is tested. Sample can be detected and analyse for the concentration of the heterocyclic hydrocarbon in the water source by using the visible light spectrophotometer. Several parameters such as pH and substrate concentration for the detection of heterocyclic hydrocarbon have been successfully indentified. Results show that all the marine bacteria used in this study are less efficient at detecting heterocyclic hydrocarbon at pH that are lower than 7, except for *Thalassospira profundimaris* which can degrade carbazole at the highest rate pH 6.5, and not able to degrade heterocyclic hydrocarbon at pH 9.5. It is also found that the system is able to detect the presence of heterocyclic hydrocarbon at a substrate concentration as low as 0.1 ppm.

Keywords: Bioremediation, Carbazole, heterocyclic hydrocarbon degrading bacteria, heterocyclic hydrocarbon

ABSTRAK

Pencemaran alam sekitar semakin teruk diakibatkan daripada pencemaran yang disebabkan oleh bahan kimia seperti hidrokarbon heterosiklik disebabkan oleh tumpahan minyak, aktiviti perindustrian dan penggunaan bahan kimia yang ditinggal pada masa yang panjang. Dalam kajian ini, kaedah pengesanan hidrokarbon heterosiklik yang berhazad menggunakan bacteria dalam sampel air telah dikaji. Sampel dianalisis tentang kepekatan hidrokarbon heterosiklik dalam sumber air telah dijalankan dengan menggunakan spektrofotometer. Beberapa parameter seperti pH dan konsentrasi substrat untuk mengesan hidrokarbon heterosiklik telah berjaya dikenalpasti. Keputusan menunjukkan bahawa semua bacteria laut yang digunakan dalam kajian ini adalah kurang berkesan untuk mengesan hidrokarbon heterosiklik pada pH yang rendah daripada 7, kecuali Thalassospira profundimaris yang boleh mendegradasikan karbazol dengan kadar tertinggi pada pH 6.5, dan tidak mampu untuk mendegradasikan hidrokarbon heterosiklik pada pH 9.5. Ia juga mendapati bahawa system ini dapat mengesan kehadiran hidrokarbon heterosiklik pada kepekatan substrat serendah 0.1 ppm.

Kata kunci: Biopemulihan, karbazol, bakteria degradasi hidrokarbon heterosiklik, hidrokarbon heterosiklik

1.0 INTRODUCTION

Heterocyclic hydrocarbons are compound that consists of at least two different kinds of elements in its ring. This compound containing nitrogen, sulphur, or oxygen have been detected in air, soil, sewage sludge, marine environments and freshwater sediments (Eisentraeger, *et al.*, 2008). The presence of large numbers of compounds in the environment will either benefits or harm to the environment. According to Eisentraeger *et al.* (2008), the environmental effects of these compound are extensively researched to understand its toxicity mechanism but still the data on its toxicity is scarce. It was found that heterocyclic hydrocarbon has the potential to cause ecotoxic (tested with algae and daphnids), cancer (tested with *Salmonella* and micosome test) and mutation to an organism if is exposed in high concentration or a long period of time.

In 1989, there were more than 200,000 barrels of crude oil spill from the oil tanker of *Exxon Valdez* in Prince William Sound, Alaska (Atlas, 1995). In addition, the recent pollution case in Mexico, the blowout of the Deepwater Horizon (DH), that has happened due to oil spillage has led to the major breakthrough in the heterocyclic hydrocarbon bioremediation and a total of 24 bacterial strains were isolated from the oiled beach and confirmed as a oil-degrading microorganisms (Kostka *et al.*, 2011). Due to these incidents, the negative environmental effects of the heterocyclic hydrocarbon were discovered. For example, it was found out that polycyclic aromatic hydrocarbon (PAH) has the ability to act as endocrine disruptor and cause the hormonal level of an organism unregulated (Swedenborg *et al.*, 2009). Due to the knowledge that has been obtain by understanding the effect that heterocyclic compounds have on the environment, it is crucial to create an efficient and cheap way for the detection of heterocyclic hydrocarbon to detect the heterocyclic hydrocarbon present in the water.

In this research, water samples that are taken from various sources in Kuching in order to detect the hazardous heterocyclic hydrocarbon. The heterocyclic hydrocarbons such as carbazole, dibenzofuran, dibenzothiophine, fluorene and biphenyl that are going to be studied because of its toxicity characteristic that is harmful to the environment. Furthermore, the detection of the heterocyclic hydrocarbon is important in order to determine the threshold level in that source. This is because if the concentration is high then treatment should have taken place to reduce the damage to the habitat of that area. In addition, this study uses marine bacteria that are able to uptake the harmful heterocyclic hydrocarbon as an energy source for its own use. Most bioremediation studies on the decomposition of harmful heterocyclic hydrocarbon use terrestrial bacteria and few of them uses marine bacteria. Therefore, the water source that is polluted with this compound can use the marine bacteria to treat or reduce the concentration.

Due to arising issue on pollution and oil spillage around the global, people are finding ways to reduce and avoid this issue. Hazardous heterocyclic hydrocarbon not only brings harm to the balance of ecosystem, but also to the health of the human being who exposed to the substances for a long period of time or in high concentration. In addition, there is need to be aware of the negative effect that it may cause such as increase the viscosity of the sea, reduce the rate of oxygen diffusion in to the sea and the health of sea organisms. If the problem is not taken care seriously, the food and water sources soon will become a problem to us as well. Furthermore, the measurement of the concentration of heterocyclic hydrocarbon in an area also need to be known and assure that it is safe to do any activity in that area by public. This is why it is very important to develop analytical methods for monitoring the presence of heterocyclic hydrocarbon in the environment. Thus, the development of a cost effective heterocyclic compounds detection system need to be done and there is a need to detect pollution level in water sample quickly in order to monitor the condition of the water source periodically.

The objectives of this study are to:

- develop a heterocyclic compound detection system using marine bacteria

- optimize the reaction for detecting heterocyclic compounds

- identify factors affecting the reaction for detecting heterocyclic compounds

2.0 LITERATURE REVIEW

2.1 Bioremediation

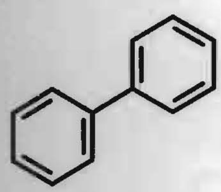
Bioremediation is a biological process that widely used to reduce or degrade the environmental pollution that is caused by human activity, accident and natural disaster. This technique of reducing the pollution is gaining its reputation for being efficient and environmentally friendly by using degrading bacteria to speed up the rate of degrading the harmful compound. However, this process required very explicit information on the types of mechanism that can be performed by the bacteria because not all the bacteria able to up the pollutants as their source and the rate of degradation is still consider slow for a large scale clean up (Atlas, 1995). Besides, in order to be able to do full scale clean-up in an area, the condition of the area need to be examined and compatible to the capability of the bacteria to adapt the environment (Balba *et al.*, 1998). Thus, many people, nowadays, use this technique for cleaning marine oil spillage although there are still many things need to be improved. Although many terrestrial bacteria that has the ability to degrade heterocyclic compound has been identified, only a few of marine bacteria was found or examined by the scientist for this aspect (Nagashima *et al,* 2009).

2.2 Marine Hydrocarbon Degrading Bacteria

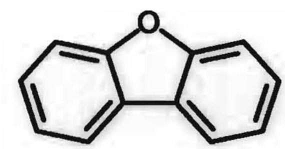
Hydrocarbon degrading bacteria present in soil and marine environment. According to Atlas (1995), the population of hydrocarbon degrading bacteria constitutes less than 1% of the total microbial community but when the presence of hydrocarbon in an area, the population of these bacteria will shoot up to 10% as compared to the total microbial community. One of the advantages of the marine hydrocarbon degrading bacteria over the terrestrial is the capability to survive in the seawater which the terrestrial cannot adapt to the conditions such as the pH, salinity, temperature changes, and water current. Besides, according to Takacs *et al.* (1964), marine bacteria are also able to maintain the osmotic pressure at the high salinity by maintain the sodium ion and potassium ions concentration at the membrane, which most of the terrestrial microbes cannot. Thus, many scientists are trying to find marine bacteria that have the ability to degrade harmful heterocyclic compound. The marine bacteria that are used are, *Pseudomonas Pachastrellae* strain M03, *Thalassospira profundimaris* strain M01 and M02, *Chromohalobacter morismortui* strain SEM01 and SEM02, and *Thalassospira Xiamenensis* strain MT01 and MT02 in this research.

2.3 Heterocyclic Hydrocarbon

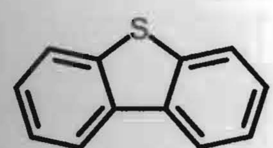
Heterocyclic hydrocarbons, such as Carbazole, Dibenzofuran, Dibenzothiophene, and Biphenyl, as shown in figure 1, are compound that consists of at least two different kinds of elements in its ring. This compound containing nitrogen, sulphur, or oxygen have been detected in air, soil, sewage sludge, marine environments and freshwater sediments (Eisentraeger *et al.,* 2008). The presence of a large number of compound in the environment will somewhat affect the surrounding, either it gives benefits or brings harm to the surrounding. The environmental effects of this compound are extensively researched to understand its toxicity mechanism but still the data on its toxicity is still scarce. Based on the research of Eisentraeger *et al.*(2008) that has been done, it was found that heterocyclic hydrocarbon has the potential to cause ecotoxic (tested with algae and daphnids), cancer (tested with *Salmonella* and micosome test) and mutation to an organism if is exposed in high concentration or a long period of time.



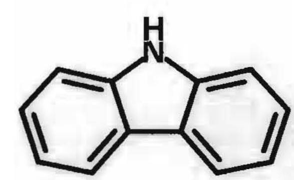
Biphenyl



dibenzofuran



Dibenzothiophene



carbazole

Figure 1: Molecular Structure of Biphenyl, Dibenzofuran, Carbazole and Dibenzothiophene

Carbazole, dibenzofuran, biphenyl and dibenzothiophene are heterocyclic hydrocarbon that can contaminate the environment. According to Bressler and Federak (2000, p.397), "In the process of biodegrading these compounds, bacteria oxidise those compounds in three most susceptible ways.

(i) the naphthalene-like attack, in which one of the aromatic rings is oxidized to a dihydrodiol

(ii) an angular dioxygenase attack, in which the carbon bonded to the methylene group in fluorene or to the heteroatoms in the analogs, and the adjacent carbon in the aromatic ring are both oxidized

(iii) the five-membered ring attack, in which the methylene carbon atom in fluorene or the sulfur atom in dibenzothiophene is oxidized."

By understanding the method that the bacteria most probably will be using to degrade the heterocyclic hydrocarbon, the intermediate and final products can be predicted much easily. Thus, we can identify the heterocyclic hydrocarbon by observing the colour changes on the medium due to the intermediate or final product. Furthermore, the concentration of the hydrocarbon present in the water sample can be determined by running the medium in spectrophotometer.

The study of Wassenberg *et al.* (2009) shows that the combination of carbazole and dibenzothiophene with the co-occurance of PAH-type aryl hydrocarbon can enhance fish embroyo toxicity although both the heterocyclic hydrocarbon is not embryotoxic itself. Thus, this proves that the presence of these compounds in the water can bring damage to the balance of ecosystem. Although dibenzothiophene is not an embryotixic, it is still the most persistent and toxic PAH in marine environment and it was found in high concentration from 22 different sampling sites in South China Sea (Yang *et al.*, 1998). According to Yang *et al.* (1998), the content of dibenzothiophene is found higher nearshore than offshore sediments and is consistent to the distribution trend of organic carbon in the sampling sites. In addition to that, it is showed that the concentration of dibenzotiophene is correlated with the organic carbon distributed in the area using linear regression (Yang *et al.*, 1998). Thus, it is reckoned that the sources of dibenzothiophene include terrestrial runoff, oil pollution and the airborne particulates formed from combustion processes (Yang *et al.*, 1998)

2.3.1 Biphenyl and Carbazole

Biphenyl is a two aromatic ring organic compound that is able to be degraded by all the marine bacteria used in this experiment. Although three enzymes are needed for the degradation of biphenyl in order to obtain its intermediate (HOPDA), yet the degradation rate of biphenyl is still much faster than degrading carbazole (Linden & Sun, 2011). This is maybe due to its molecular and physical properties of the compound. Although biphenyl is insoluble in water, the hydrophobic properties of biphenyl are not as strong as carbazole. This can be seen when same amount of carbazole and biphenyl is added into the water, but carbazole become a huge precipitate while biphenyl only insoluble in the form of powder in water. This allows larger TSA/V for the bacteria to come in contact with biphenyl rather than carbazole.

Although the degradation of carbazole only needs two enzyme to obtain the intermediate product, HOADA, the molecular structure and the physical properties of carbazole makes it hard for the enzyme to come in contact or approach the heterocyclic hydrocarbon (Trinh, 2012). The insoluble in water properties of carbazole might also cause the reduction of the chance of bacteria to come in contact with carbazole (Bastiaens *et al.*, 2000). The bacteria might also need to figure a way to identify the conpound and degrades it. In addition to that, carbazole has three aromatic rings that are arrange in such a way that it is very stable and the bond are hard to be broken (Watts, 1998). Moreover, the more aromatic ring presence in a compound, the harder it is for it to be degraded.